Claims

1. Method for the quantification of tumor cells in a body fluid, characterized in that
(a) the sample to be investigated is subjected to a method for concentrating or depleting tumor cells and
(b) a reaction is carried out, on the concentrated or depleted tumor cells, in which the mRNA coding for the catalytic subunit of telomerase is specificially amplified, and
(c) the amount of amplified nucleic acid is determined quantitatively.

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2. Method according to Claim 1, characterized in that a reverse transcription reaction in which the mRNA contained in the sample is transcribed into cDNA is carried out before the amplification reaction with the sample to be investigated.

3. Method according to Claims 1 or 2, characterized in that a DNase reaction is carried out with the sample to be investigated before the transcription of the mRNA into cDNA.

4. Method according to any of Claims 1 - 3, characterized in that the sample to be investigated is purified, preferably by an ion exchange chromatography, in particular on silica gel.

5. Method according to any of Claims 1 - 4, characterized in that, for quantitative determination of the telomerase-coding nucleic acid, the amplification products are labeled even during amplification and the amplification kinetics are measured continuously even during the amplification process.

6. Method according to Claim 5, characterized in that a probe which is specific for the amplification products, and which emits a characteristic signal proportional to the products amplified per synthesis cycle, is present during amplification.

7. Method according to any of Claims 1 - 4, characterized in that, for quantitative determination

of the telomerase-encoding nucleic acid, at least one, preferably three, standard nucleic acids are coamplified and are added in different concentrations to the sample to be investigated.

8. Method according to any of Claims 1 - 7, characterized in that the amplification product is quantified either directly or via a label, preferably via a radioactive label, a biotin label, a fluorescent label or an electrochemoluminescent label.

9. Method according to any of Claims 1 - 7, characterized in that the amplification product is detected via a hybridization with a labeled oligonucleotide, where the label is preferably a radioactive label, a biotin label, a fluorescent label or an electrochemoluminescent label.

10. Method according to any of Claims 7 - 9, characterized in that, to quantify the telomerase-encoding nucleic acid to be determined, the amount of coamplified nucleic acid or nucleic acids is compared with the amount of telomerase-encoding nucleic acid.

11. Method according to any of Claims 1 - 10, characterized in that the sample to be investigated is peripheral blood, and in that a reaction is carried out with the sample to be investigated as positive control, in which a nucleic acid which occurs in peripheral blood, preferably the mRNA coding for β-globin, glyceraldehyde-phosphate dehydrogenase, β-actin or the T-cell receptor, is specifically amplified and detected.

12. Method according to Claim 1 or any of Claims 3 - 11, characterized in that, as negative controls, no reverse transcription reaction is carried out before the amplification reaction with the sample to be investigated and/or water is employed in place of the body fluid.

13. Method according to any of Claims 1 - 12, characterized in that the following oligonucleotide primers are used for the amplification:

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5' CTACCGGAAG AGTGTCTGGA GCAAGTTGCA AAGC 3' (hTRT1)
and/or
5' GGCATACCGA CGCACGCAGT ACGTGTTCTG 3' (hTRT2),

where hTRT1 and/or hTRT2 comprises where appropriate a promoter sequence for an RNA polymerase.

14. Method according to any of Claims 1 - 13, characterized in that a DNA polymerase or an RNA polymerase is used for the amplification.

15. Method according to any of Claims 1 - 14, characterized in that, in the case of amplification with DNA polymerase, the polymerase chain reaction (PCR) is carried out and, in the case of amplification with RNA polymerase, the isothermal nucleic acid sequence-based amplification (NASBA) is carried out.

16. Method according to any of Claims 1 - 15, characterized in that the sample to be investigated is blood, and in that the blood sample to be investigated is depleted in ~~step~~ stem cells and/or activated immune cells, preferably by immunoabsorption.

17. Method according to any of Claims 1 - 16, characterized in that the sample to be investigated is blood, and the tumor cells from the blood sample to be investigated are concentrated, preferably by immunoabsorption.

18. Method according to any of Claims 1 - 17, characterized in that the cells contained in the sample are cultivated under conditions which are unfavorable for telomerase-positive nontumor cells but favorable for the tumor cells present.

19. Method according to Claim 18, characterized in that the duration of the cultivation is such that nontumor cells die and tumor cells survive.

20. Method according to any of Claims 1 - 19, where, for concentrating the tumor cells, a cell separation medium is covered with a layer of the body fluid and centrifuged, characterized in that the cell separation medium has a density in the range from 1.055 to < 1.070 g/ml.

21. Method according to Claim 20, characterized in that the cell separation medium has a density in the range from 1.060-1.067 g/ml and preferably of about 1.065 g/ml.

22. Method according to Claim 20 or 21, characterized in that the centrifugation is carried out at about 1000 × g for about 30 minutes.

23. Method according to any of Claims 20 - 22, characterized in that the cell separation medium used is Percoll or Ficoll.

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24. Method according to any of Claims 20 - 23, characterized in that the body fluid is, prior to being applied as a covering layer, admixed with one or more substances which prevent aggregation of platelets to tumor cells, and/or the body fluid is, prior to being applied as a covering layer, freed of substances which promote aggregation of platelets to tumor cells.

25. Method according to any of Claims 20 - 24, characterized in that the body fluid is peripheral blood.

26. Method according to Claim 25, characterized in that the peripheral blood is drawn in an anticoagulant substance and, prior to covering the cell separation medium, diluted with a diluent, preferably in a ratio of about 1:1.

27. Method according to Claim 25 or 26, characterized in that the peripheral blood is venous or arterial blood.

28. Method according to any of Claims 20 - 24, characterized in that the body fluid is selected from lymph, urine, exudates, transudates, spinal fluid, seminal fluid, saliva, fluids from natural or unnatural body cavities, bone marrow and dispersed body tissue.

29. Method according to any of Claims 20 - 28, characterized in that the centrifugation vessel is, after centrifugation and before the tumor-cell-enriched interphase is removed, cooled intensively to prevent mixing of the cells in the different layers.

30. Method according to any of Claims 20 - 29, characterized in that the centrifugation is carried out in a vessel which is divided by a porous barrier, a filter or a sieve into an upper and a lower compartment, where the cell suspension medium is initially charged in the lower compartment and the body fluid is introduced into the upper compartment.

31. Method according to Claim 30, characterized in that the porous barrier, the filter or the sieve have [sic] a thickness of 1-10 mm, preferably about 5 mm.

32. Method according to Claim 30 or 31, characterized in that the porous barrier, the filter or the sieve have [sic] a pore size of 20-100 µm, preferably 20-30 µm.

33. Method according to any of Claims 30 - 32, characterized in that the porous barrier, the filter or the sieve are [sic] made of a hydrophobic material or coated with a hydrophobic material.

34. Method according to any of Claims 20 - 33, characterized in that the cell separation medium contains a dye which makes the color of the cell separation medium distinguishable from that of the supernatant body fluid, thus simplifying the localization of the interphase.

35. Method according to any of Claims 1 - 34, characterized in that the sample to be investigated is blood, and in that there is an investigation in said method of, on the one hand, a venous blood sample and, on the other hand, an arterial blood sample, and the results are compared with one another.

36. Method according to any of Claims 1 - 35, characterized in that the sample to be investigated is blood, and in that there is an investigation in said method of, on the one hand, a blood sample from the finger pad and, on the other hand, a venous or arterial blood sample, and the results are compared with one another.

37. Method according to any of Claims 1 - 36, characterized in that the tumor cells are derived from

metastases, preferably micrometastases, of malignant tumors.

38. Method according to any of Claims 1 - 37, characterized in that the tumor cells are selected from a group of cells of metastasizing tumors and/or neoplasms which are derived from a T-cell lymphoblastoma, T-cell leukemia cells, chronic myeloid leukemia cells, acute lymphatic leukemia cells, chronic lymphatic leukemia cells, teratocarcinoma, melanoma, carcinoma of the lung, large intestine cancer, breast cancer, hepatocellular carcinoma, kidney tumor, adrenal tumor, prostate carcinoma, neuroblastoma, brain tumor, rhabdomyosarcoma, leiomyosarcoma and/or lymphoma.

39. Oligonucleotide primer with the sequence

5' CTACCGGAAG AGTGTCTGGA GCAAGTTGCA AAGC 3' (hTRT1)
and/or
5' GGCATACCGA CGCACGCAGT ACGTGTTCTG 3' (hTRT2),

where hTRT1 and/or hTRT2 may, if appropriate, additionally comprise a promoter sequence for an RNA polymerase.

40. Oligonucleotide probe with the sequence

5' CGTTCTGGCT CCCACGACGT AGTC 3' (hTRT o)

and/or the corresponding reverse complementary sequence thereof.

41. Kit for the quantification of tumor cells in a body fluid, comprising:
(a) an oligonucleotide primer pair for specific amplification of telomerase-encoding nucleic acid.

42. Kit according to Claim 41, characterized in that the oligonucleotide primer pair specified in (a) has the following sequences:

5' CTACCGGAAG AGTGTCTGGA GCAAGTTGCA AAGC 3' (hTRT1)
and/or
5' GGCATACCGA CGCACGCAGT ACGTGTTCTG 3' (hTRT2),

where hTRT1 and/or hTRT2 comprises where appropriate a promoter sequence for an RNA polymerase.

43. Kit according to either of Claims 41 or 42, characterized in that it additionally comprises (b) a standard nucleic acid or standard nucleic acids for coamplification.

44. Kit according to any of Claims 41 - 43, characterized in that it additionally comprises a labeled oligonucleotide for detecting the amplified nucleic acid of the sample to be determined and/or one or more labeled oligonucleotides for detecting the coamplified standard nucleic acid or standard nucleic acids, in particular an oligonucleotide with the sequence:

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5' CGTTCTGGCT CCCACGACGT AGTC 3' (hTRT o)

and/or the corresponding reverse complementary sequence thereof.

45. Kit according to any of Claims 41 - 44, characterized in that it additionally comprises a reverse transcriptase, a DNA polymerase, preferably a Taq polymerase, a DNase and/or suitable buffers and, where appropriate, labeled nucleotides and, where appropriate, means suitable for the depletion of stem cells and/or activated immune cells and/or for the concentration of tumor cells.

46. Kit according to any of Claims 41 - 45, characterized in that it additionally comprises a reverse transcriptase, an RNA polymerase, preferably a T7 RNA polymerase, an RNase H, a DNase and/or suitable buffers and, where appropriate, labeled nucleotides and, where appropriate, means suitable for the depletion of stem cells and/or activated immune cells and/or for the concentration of tumor cells.

47. Kit according to any of Claims 41 - 46, characterized in that it additionally comprises a cell separation medium having a density in the range of from

1.055 to < 1.070 g/ml and, if appropriate, a centrifugation vessel.

48. Kit according to Claim 47, characterized in that the cell separation medium has a density in the range of from 1.060 to 1.067 g/ml and preferably of about 1.065 g/ml.

49. Kit according to either of Claims 47 or 48, characterized in that the centrifugation vessel has a porous barrier, a filter or a sieve of a thickness of 1-10 mm, preferably of about 5 mm, which divide [sic] the centrifugation vessel into an upper and a lower compartment.

50. Kit according to Claim 49, characterized in that the porous barrier, the filter or the sieve have [sic] a pore size of 20-100 μm, preferably 20-30 μm.

51. Kit according to Claim 49 or 50, characterized in that the cell separation medium is in the lower compartment of the centrifugation vessel.